Neuroendocrine Regulation of GnRH Release
in Induced Ovulators

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GnRH is the key neuropeptide controlling reproductive function in all vertebrate species. Two different neuroendocrine mechanisms have evolved among female mammals to regulate the mediobasal hypothalamic (MBH) release of GnRH leading to the preovulatory secretion of LH by the anterior pituitary gland. In females of spontaneously ovulating species, including rats, mice, guinea pigs, sheep, monkeys, and women, ovarian steroids secreted by maturing ovarian follicles induce a pulsatile pattern of GnRH release in the median eminence that, in turn, stimulates a preovulatory LH surge. In females of induced ovulating species, including rabbits, ferrets, cats, and camels, the preovulatory release of GnRH, and the resultant preovulatory LH surge, is induced by the receipt of genital somatosensory stimuli during mating. Induced ovulators generally do not show "spontaneous" steroid-induced LH surges during their reproductive cycles, suggesting that the positive feedback actions of steroid hormones on GnRH release are reduced or absent in these species. By contrast, mating-induced preovulatory surges occasionally occur in some spontaneously ovulating species. Most research in the field of GnRH neurobiology has been performed using spontaneous ovulators including rat, guinea pig, sheep, and rhesus monkey. This review summarizes the literature concerning the neuroendocrine mechanisms controlling GnRH biosynthesis and release in females of several induced ovulating species, and whenever possible it contrasts the results with those obtained for spontaneously ovulating species. It also considers the adaptive, evolutionary benefits and disadvantages of each type of ovulatory control mechanism. In females of induced ovulating species estradiol acts in the brain to induce aspects of proceptive and receptive sexual behavior. The primary mechanism involved in the preovulatory release of GnRH among induced ovulators involves the activation of midbrain and brainstem noradrenergic neurons in response to genital–somatosensory signals generated by receipt of an intromission from a male during mating. These noradrenergic neurons project to the MBH and, when activated, promote the release of GnRH from nerve terminals in the median eminence. In contrast to spontaneous ovulators, there is little evidence that endogenous opioid peptides normally inhibit MBH GnRH release among induced ovulators. Instead, the neural signals that induce a preovulatory LH surge in these species seem to be primarily excitatory. A complete understanding of the neuroendocrine control of ovulation will only be achieved in the future by comparative studies of several animal model systems in which mating-induced as well as spontaneous, hormonally stimulated activation of GnRH neurons drives the preovulatory LH surge. KEY WORDS: GnRH; MBH; induced ovulators; neuroendocrine regulation; preovulatory LH surge; spontaneous ovulators. © 2000 Academic Press


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INTRODUCTION

Females of induced ovulating mammalian species (e.g., voles, rabbits, mink, ferrets, cats, and camels) undergo periods of behavioral estrus when ovulation is induced by sensory stimulation associated with mating. By contrast, females of spontaneously ovulating species (e.g., rats, hamsters, guinea pigs, sheeps, monkeys, and humans) undergo ovarian cycles in which ovulation is induced by ovarian steroid hormones at regular intervals. Many have argued that this classification of species into either "induced" or "spontaneous" ovulators is too rigid (46, 104, 152, 251). There are numerous examples of coitus-induced or coitus-facilitated ovulation in spontaneously ovulating species. The laboratory rat and mouse are probably the best examples of species in which both ovulatory mechanisms appear to be integrated. The female rat must receive intromissive stimulation in order for the ovaries to develop fully functional corpora lutea. Mating stimulates the release of prolactin from the anterior pituitary which is necessary for luteal activity (reviewed in 212). In addition, female rats exhibiting persistent vaginal estrus either spontaneously at middle age (69) or after exposure to constant light (56) may show coitus-induced ovulation. The spontaneously mutant *hpg* mouse (37), which bears a specific deletion in the GnRH gene, showed mating-induced but not spontaneous ovulation when brain grafts containing GnRH neurons were implanted into the third ventricle (77). In cattle (141, 204), mating during the first 6–8 h of behavioral estrus advanced the preovulatory LH surge and resultant ovulation. Taken together, these observations suggest that the neural circuitry underlying the reflexive release of LH is present in some spontaneously ovulating species. By contrast, induced ovulating species generally do not show steroid-induced preovulatory LH surges. Regimens of estradiol treatment, either with or without concurrent progesterone, that successfully induced LH surges in rats failed to stimulate LH secretion in rabbits (197), voles (149, 150), or ferrets (12). The cat seems to be an exception to this rule in which spontaneous ovulations have been observed occasionally in group-housed females (85, 123). Taken together, the available data suggest that positive feedback actions of steroid hormones on gonadotropin release are either reduced or absent in induced ovulating species.

This review will summarize what is known about the neuroendocrine mechanisms controlling the preovulatory LH surge among induced ovulating species. These mechanisms involve ovarian steroid hormones, sensory inputs resulting from mating, the forebrain GnRH neurons and the network of neurons which control the release of GnRH into the pituitary portal vessels, and the pituitary gonadotrophs, which respond to GnRH by releasing LH. In addition, we will discuss some of the main differences and similarities between the neuroendocrine mechanisms controlling induced versus spontaneous ovulation.
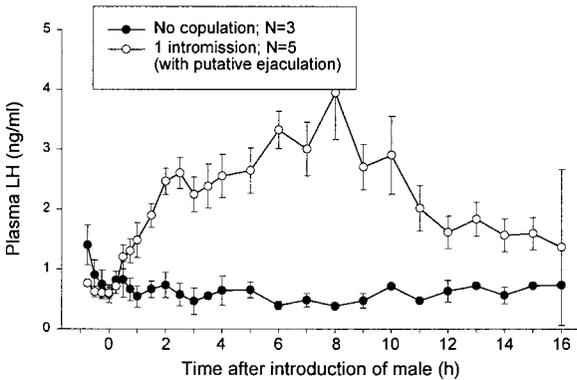
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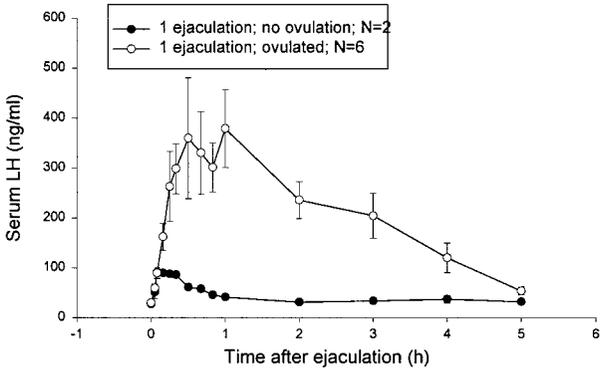
Adapted from Jones et al. (Fertility and Sterility 1976; 27: 848-852).

Cat
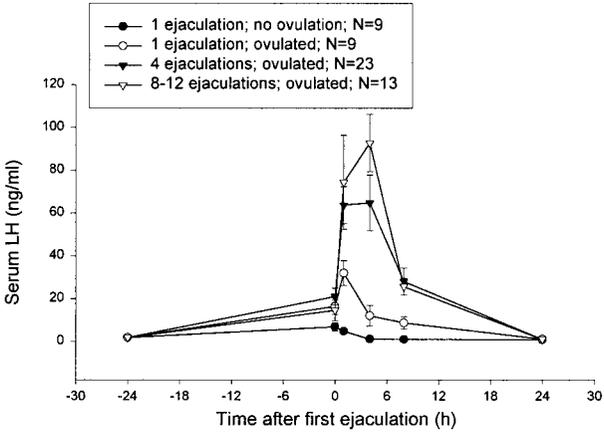
Adapted from Concannon et al. (Biol Reprod 1980; 23: 111-117)

Ferret

Adapted from Carroll et al. (Biol Reprod 1985; 32: 925-933)

CHARACTERISTICS OF THE LH SURGE

The Preovulatory LH Surge

The primary endocrine response to coitus in induced ovulators is an immediate release of LH from the anterior pituitary that results in ovulation. There is some species variability in the pattern of mating behavior preceding a preovulatory surge in LH and in the latency, duration, and magnitude of this LH peak after mating. To date, the coitus-induced LH surge has been characterized in rabbits (*Oryctolagus cuniculus;* 64, 80, 105), voles (*Microtinae;* 38), cats (*Felis catus;* 47, 107, 238), ferrets (*Mustela furo;* 33), and camels (*Camelus dromedarius;* 140). However, the relationship between the amount of mating stimulation received and the resultant preovulatory LH surge has been studied systemically only in cats (47, 107, 238) and ferrets (33). Typically, the postcoital rise in LH can be defined either as an "all-or-none" response to the first effective stimulus or as a more cumulative response to the amount of mating stimulation received. In the ferret (33), a single intromission induced a prolonged preovulatory LH surge in the estrous female. This elevation in plasma LH levels began around 1.5 h after the onset of intromission, reached a maximum approximately 6 h later, and was sustained for at least 12 h (33; Fig. 1). Even though intromission duration varied from 1 to 94 min, ovaries of every ferret receiving an intromission had corpora lutea 1 week later. Thus, the preovulatory surge in LH in the ferret seems to be independent of the amount of mating stimulation received and is therefore an all-or-none phenomenon. Likewise, in the mink (*Mustela vision;* 67), ovulation can be induced by receipt of an intromission as short as 2 min. Although no data are currently available on plasma LH levels after mating in this species, a single intromission presumably suffices to induce a preovulatory LH surge. In the rabbit (105), a short mating bout including ejaculation induces a rapid release of LH from the pituitary. Plasma LH levels started to rise within 3 min after receipt of an ejaculation and reached a plateau within 15 min (105; Fig. 1). By contrast, in the cat (47, 107, 238) the duration and magnitude of the LH surge are related to the amount of coital stimulation received (Fig. 1). In cats (107), plasma LH levels increased rapidly after a single mating and peaked 20 min later. The pattern of the single mating-induced LH release was very similar to that occurring after a single injection of GnRH (106). However, a single mating bout induced ovulation in only 50% of the females studied (47, 238). When female cats were allowed

FIG. 1. Changes in serum or plasma LH levels following receipt of intromission with ejaculation in the rabbit, cat, and ferret. In both rabbit and ferret, the magnitude of the preovulatory LH surge is independent of the amount of mating stimulation received. By contrast, in the cat the duration and magnitude of the LH surge is related to the amount of mating stimulation received. Data are represented as means ± SEM.

to receive multiple ejaculations or to mate at their own pace, plasma LH levels were significantly higher compared to those of once-mated cats, and all cats ovulated (47, 107, 238). Therefore in contrast to ferrets and minks, in cats repeated copulations probably trigger episodes of GnRH release, thereby producing cumulative increments in plasma LH levels.

Stimulus Requirements for Inducing a Preovulatory LH Surge

In most induced ovulating species, stimulation of the female's genital tract by penile intromission from a male conspecific most effectively induces the preovulatory release of LH from the pituitary, although there are reports (1, 67) of ovulation occurring in female mink after brief exposure to a male in the absence of actual intromission. The amount of mating stimulation required to induce a preovulatory LH surge is usually much less than what is normally provided during copulation. For example, voles (57, 152), rabbits (158), and cats (23) all copulate in bouts which involve multiple intromissions and ejaculations. Despite this extensive copulatory activity, the effective stimulus for inducing ovulation is just a single intromission in *Microtus agrestis* (148) and a single ejaculatory series in *Microtus montanus* (116). Likewise, in rabbits (158), alpacas (73), and racoons (233), only one ejaculation was required to induce ovulation in the majority of animals. By contrast, female prairie voles (*Microtus ochrogaster;* 34) and cats (47, 107, 238) must receive multiple intromissions and ejaculations in order to ovulate.

Shrews (Order Insectivora) are the only induced ovulating species known to require extensive mating stimulation over a period of at least 24 h to induce ovulation. When female musk shrews (*Suncus murinus*) were mated for less than 4 h, only one third of the females ovulated (181). However, when female musk shrews received multiple intromissions and ejaculations over at least 24 h, all animals ovulated (55). Likewise, in the least shrew (*Cryptotis parva;* 154), copulatory activity lasting 12–36 h was needed to induce ovulation. Thus the reproductive physiology of shrews does not seem to resemble that of classic induced ovulating species, in which mating readily induces a preovulatory LH surge leading to ovulation.

Evolution of Induced Ovulation

The occasional occurrence of induced ovulation in species generally known as spontaneous ovulators inspired the hypothesis (46, 251) that induced ovulation is a primitive characteristic from which spontaneous ovulation has evolved. Jöchle (104) extended this concept even further by proposing that all mammalian species, including human, can show coitus-induced ovulation on specific (usually adverse) occasions ("facultative-induced ovulators"). This would suggest that this primitive trait has been conserved among higher
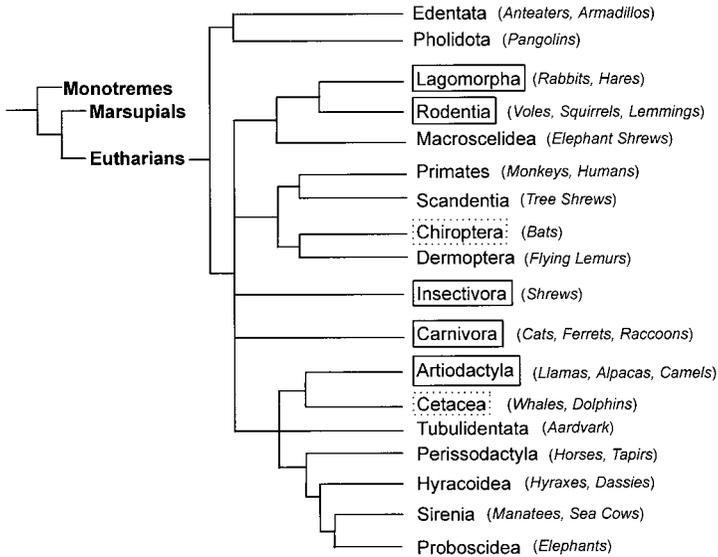
FIG. 2. Phylogenetic distribution of induced ovulation among mammalian species. Induced ovulating species have been found among the Orders Lagomorpha, Rodentia, Insectivora, Carnivora, and Artiodactyla (solid boxes). Additional species that have been suggested to be induced ovulators belong to the Orders Chiroptera and Cetacea (dotted boxes).

mammals. It should be noted that the occurrence of coitus-induced ovulation and conception in women is mostly based on circumstantial evidence such as reports of pregnancies in victims of rape and women with amenorrhoea, and thus is quite controversial (103, 104). One could argue that rape victims who become pregnant are more likely to report the crime to the police than those who do not.

The widespread occurrence of induced ovulation in the primitive Order Insectivora and in Rodentia supports the notion that induced ovulation is a primitive trait (46, 104, 251). Zarrow and Clarke (251) further argued that its occurrence in Insectivora, Lagomorpha, and Rodentia reflects the relatively close phylogenetic relationship among these orders and that the occurrence of induced ovulation in some Carnivores reflects its early separation from the Ungulates (Fig. 2). However, this cannot explain the occurrence of induced ovulation in the Camelidae. Also, if induced ovulation is a primitive trait, one would expect it to be the prevailing ovulatory mechanism among nonmammalian vertebrates, such as fish, amphibians, reptiles, and birds, as well as among the more primitive mammals, the monotremes and marsupials. This is not the case. Generally, nonmammalian vertebrates show "spontaneous" steroid-induced ovulation. Only the red-sided garter snake (145) has been identified as an induced ovulator among nonmammalian vertebrates. In addition, there is one report (6) suggesting that the marsupial species *Potorous tridactylus* is an induced ovulator. It should be noted, however, that our knowledge of the reproductive strategies of most vertebrate species is still very limited.

It has been suggested (70, 152) that induced ovulation evolved on at least two separate occasions. This trait probably evolved after the evolutionary lines of the marsupials and eutharians separated during the early Cretaceous period (>100 million years ago; Fig. 2). Induced ovulation may have evolved as a special case of male-induced activation of the GnRH neuronal system in females of several species. There are numerous examples among mammals (reviewed in 26, 189, 234), birds (reviewed in 98, 127), and reptiles (reviewed in 49) in which the display of behaviors by a male stimulates gonadotropin secretion and consequent ovarian development and sexual behavior in female conspecifics. For instance, introducing males into a group of seasonally anestrous female goats induced estrous cycles and subsequent (spontaneous) ovulation (39). This "male-effect" in goats most likely reflects a pheromone-induced activation of GnRH release (87). Similarly, pheromonal cues derived from males induce estrus in female mice (142) and female gray short-tailed opossums (*Monodelphis domestica;* 71). Another example of activation of the reproductive neuroendocrine system by male cues is the increase in number of female whiptail lizards (*Cnemidophorus inornatus*) ovulating when a sexually active conspecific male is present (48). Interestingly, the same is true for the unisexual *Cnemidophorus uniparens* in the presence of a female showing male-typical sexual behavior (48). The latter emphasizes the evolutionary significance of exposure to a male for successful breeding and thus a positive selection pressure for maintaining the expression of male-typical behaviors among females.

A positive selection pressure for induced ovulation may be the resultant optimal synchronization of ovulation in the female with the provision of sperm by the male, thereby preventing any evolutionary costs associated with wastage of gametes by females. If this is true, it is surprising that induced ovulation has not evolved as the prevailing ovulatory mechanism among all vertebrates. The evolutionary disadvantage of induced ovulation might involve the absence of sperm competition, i.e., the competition between sperm ejaculated by different male partners for fertilization of a female's ova (165). Initially, sperm competition was viewed largely from a male perspective, i.e., the battle between males to mate with passively receptive females (165). It is now clear that females also play an important role in mate selection by actively choosing whether or not to mate with certain males. This suggests that females promote sperm competition because they benefit from it as well. For instance, by promoting sperm competition, females enhance the genetic quality of their offspring—on the presumption that genetically superior males produce sperm that compete most effectively in situations with sperm competition (79). Sperm competition is widespread across the animal kingdom (207), although it has been studied most extensively in *Drosophila* (175) and zebra finches (reviewed in 21). In insects and birds, the last male to inseminate a female before she ovulates usually fertilizes the majority of her ova. Like spontaneous ovulators, females of induced ovulating species actively choose their mating partners. For example, female musk shrews rejected certain males but were receptive to other males which were introduced immediately before or after the rejected

males (63). However, the time window between mating and the occurrence of ovulation in induced ovulators is probably too narrow to permit appreciable sperm competition in the female's reproductive tract (79). Although there is some variation in the interval between mating and ovulation among induced ovulating species, ovulation and fertilization typically occur within 24 h after mating in most species (152). Thus the first male to ejaculate with a female will usually fertilize her ova. In accordance with this first-male advantage, field data (202) from the 13-lined ground squirrel (*Citellus tridecemlineatus*) showed that males that encountered already mated females tended not to mate with them. A prolongation of behavioral estrus after mating, which occurs in some induced ovulators, may lead to matings with additional males which, in turn, could lead to some sperm competition. In ferrets (219), voles (36), and cats (236), circulating progesterone levels do not rise until 3–4 days after mating which is when females' proceptivity and receptivity decline. In cats (236), repeated matings elicit a LH response only during the first 2 days of estrus even though estrus lasts 6 days on average. Taken together, these data suggest that there is probably very limited sperm competition in induced ovulating species. The absence of sperm competition may counterbalance the evolutionary advantage of synchronization of gamete availability that prevails in induced ovulating species. As a result, induced ovulation evolved in only a limited number of mammalian species.

Ovarian Control of Female Sexual Receptivity

Female reproduction requires a finely tuned coordination of the brain, pituitary, and ovaries to produce mature ova. The developing ovarian follicles secrete high levels of estradiol which act in the hypothalamus to promote the display of female sexual behavior, thereby ensuring that the female is maximally receptive to a male conspecific at the time that she is ready to ovulate. There is some species variability in the duration and periodicity of estrus among induced ovulators (Table 1). Caution is warranted in interpreting the data in Table 1 since information on estrus duration and the interval between mating and ovulation is still scarce for many induced ovulators. Most induced ovulating species show well-defined periods of behavioral estrus which last a few days. For instance, female cats remain receptive for an average of 6 days during which mating may occur repeatedly (237). By contrast, female ferrets will stay in estrus for up to several months or until mating occurs (88, 143, 186).

The duration and occurrence of estrous periods are strongly influenced by environmental and social cues. Many mammals are seasonal breeders, with reproductive activity being controlled by seasonal changes in photoperiod. For instance, the ferret comes into estrus in response to lengthening days (22) which occur in early spring in the northern hemisphere. Males show testicular development at the same time. If mated, females become pregnant, deliver a litter approximately 41 days later, and nurse the litter for an additional 42

TABLE 1

List of Mammalian Species Showing Mating-Induced Ovulation (Adapted from 152)

Order	Species	Estrus characteristics	Mating requirements for ovulation	Seasonal breeder	Interval between mating and ovulation
Insectivora	Short-tailed Shrew (*Blarina brevicauda*)	Estrus duration: up to 33 days in the absence of a male	Multiple ejaculatory series	Yes	55–71 h (64 h on average)
	Least Shrew (*Cryptotis parva*)	Estrus duration: 1–2 days	Multiple ejaculatory series for at least 12–36 h	No	?
	White-toothed Shrew (*Crocidura russula monacha*)	No ovarian estrous cycles; receptive throughout pregnancy	?	No	15–17 h
	Asian Musk Shrew (*Suncus murinus*)	No ovarian estrous cycles; estrus is induced by exposure to males; receptive throughout pregnancy	Multiple ejaculatory series for at least 24 h	Yes	19 h
Rodentia	13-lined ground squirrel (*Citellus tridecemlineatus*)	Estrus duration: 2–4 weeks, recurring at 2–4 week intervals	Single ejaculatory series	Yes	8–12 h
	5-striped Indian palm squirrel (*Funambulus pennanti*)	Estrus duration: 1 day	?	Yes, depending on °N	24 h
	Short-tailed field vole (*Microtus agrestis*)	Estrus duration: 1–5 days; recurring at irregular intervals	Single intromission	Yes	9–12 h
	California mouse (*Microtus californicus*)	?	?	No	<15 h
	Montane vole (*Microtus montanus*)	?	Single ejaculatory series	Yes	8–10 h
	Prairie vole (*Microtus ochrogaster*)	Estrus is induced by exposure to males	Multiple ejaculatory series	No	10–11 h
	Meadow vole (*Microtus pennsylvanicus*)	?	?	Yes, depending on °N	?
	Pine vole (*Microtus pinetorum*)	?	?	?	?
	Tree mouse (*Phenacomys longicaudus*)	Estrus duration: up to 21 days (average 5.9 days)	?	Yes	?
	Collared lemming (*Dicrostonyx groenlandicus*)	Estrus duration: 2–22 days	?	No	9–10 h
Lagomorpha	Hare (*Lepus europaeus*)	Estrus duration: up to 14 days; prepartum estrus	Single ejaculatory series	Yes	<24 h
	Rabbit (*Oryctolagus cuniculus*)	Estrus duration: 2–15 days; recurring at 4–6 day intervals	Single ejaculatory series	No	11–12 h

TABLE 1—*Continued*

Order	Species	Estrus characteristics	Mating requirements for ovulation	Seasonal breeder	Interval between mating and ovulation
Carnivora	Domestic cat (*Felis catus*)	Estrus duration: 6 days on average	Multiple ejaculatory series	Yes	24–54 h
	Ferret (*Mustela furo*)	Estrus duration: up to 17 weeks	Single intromission	Yes	30 h
	Mink (*Mustela vison*)	Estrus duration: up to 3–4 weeks	Single intromission	Yes	33–72 h
	Racoon (*Procyon lotor*)	Estrus duration: 2–3 days	Single ejaculatory series	Yes	?
Artiodactyla	Llama (*Lama glama*)	?	?	Yes	24–48 h
	Alpaca (Lama pacos)	Estrus duration: 4–36 days	?	Yes	
	One-humped Camel (*Camelus dromedarius*)	Estrus duration: 17–28 days	Single ejaculatory series	Yes	12–24 h
	Two-humped Camel (*Camelus bactrianus*)	Estrus duration: 1–7 days; recurring at 10–20 day intervals	?	No	?

°N: north latitude

days. After weaning their young, females usually come back into estrus and become pregnant again if mated. Generally, female ferrets will breed only twice a year. In some other induced ovulators social cues play an essential role in activating the reproductive axis. For example, in virgin female prairie voles, estrus is induced by exposure to an unfamiliar male (reviewed in 180). Pheromonal and tactile stimuli from a male caused significant increases in uterine weights (35) and circulating estradiol levels (44). As in voles, behavioral receptivity in musk shrews depends on the presence of a male (63, 185). Female musk shrews typically show no ovarian cycles. When she first encounters a male, the female shrew initially responds very aggressively for up to 1 h. Eventually the female becomes sexually receptive and permits the male to copulate with her. Females continue to be receptive to males for several days (reviewed in 201). This response of behavioral receptivity toward the male is too rapid to be stimulated by ovarian estradiol production, because circulating estradiol levels are relatively low around the time of mating (75). Females' sexual behavior is probably stimulated by testosterone from adrenal origin, which acts in the hypothalamus after being aromatized to estradiol (182).

In induced ovulating species, estradiol is the sole endocrine stimulus acting in the brain to promote behavioral estrus. Administration of estradiol induced full behavioral receptivity in ovariectomized rabbits (18), ferrets (15), cats (231), prairie voles (59), collared lemmings (*Dicrostonyx groenlandicus;* 92), and brown lemmings (*Lemmus trimucronatus;* 102). Likewise, estradiol treat-

ment to female musk shrews activated sexual behavior, though only when administered in supraphysiological doses (183). Testosterone appears to be more effective than estradiol in activating behavioral receptivity in ovariectomized musk shrews (182). There is no indication that progesterone is also needed for the induction of behavioral receptivity in induced ovulators that have been estrogen primed. Short-term (4–8 h) exposure to progesterone of ovariectomized females primed with low doses of estradiol did not affect behavioral receptivity in brown lemmings (102) or ferrets (13). Long-term (>24 h) exposure to progesterone clearly inhibited female receptivity in these species (13, 102). Likewise, in the prairie vole (59), administration of progesterone to estradiol-primed animals induced both short-term (within hours) and long-term inhibition of sexual responses. This contrasts with the situation in many spontaneous ovulators, such as rats (160), hamsters (53), guinea pigs (254), and goats (20), in which progesterone has both facilitatory and inhibitory effects on sexual receptivity depending on when it is administered.

Progesterone binding sites have been found in the preoptic area (POA), mediobasal hypothalamus (MBH), amygdala, anterior pituitary, and some cortical areas of ovariectomized female ferrets (13). Their distribution resembles that previously reported for progesterone receptors in the rat (138, 157) and guinea pig (23). As in numerous spontaneously ovulating species, including rats (138, 157), guinea pigs (23), hamsters (124), mice (187), and macaques (137), exposure to estradiol significantly enhanced the concentration of cytosolic progesterone binding sites in the hypothalamus and pituitary gland of ovariectomized female ferrets (13). In contrast to rats (138, 157) and guinea pigs (23), estradiol treatment did not significantly augment progesterone binding sites in the ferret's POA. Likewise, in the prairie vole (45), in which estrus is induced by exposure to male-derived stimuli, progesterone receptor binding was augmented in the MBH, but not the POA, when females were exposed to a male for 18 h followed by 30 h alone in the male's soiled cage. Thus, estradiol clearly stimulates synthesis of nuclear progesterone receptors in both induced and spontaneous ovulators. However, there are differential effects of progesterone on female sexual receptivity between induced and spontaneous ovulators. It should be noted, however, that the absence of a facilitatory effect of progesterone on female's sexual receptivity is not limited to induced ovulating species. For example, progesterone did not facilitate the expression of female sexual behavior in ovariectomized, estradiol-primed rhesus monkeys (14). Likewise, estradiol failed to stimulate the synthesis of cytosolic progesterone receptors in the POA of monkeys (137). Whether differences in the distribution of estrogen-induced progesterone receptors underlie this differential effect of progesterone on female sexual behavior in induced versus spontaneous ovulators remains questionable.

## GnRH CONTROL OF THE PREOVULATORY LH SURGE

The decapeptide GnRH is the primary hypothalamic regulator of LH release in both spontaneous and induced ovulators. GnRH, or LH-RH as it was

originally called, was discovered independently in the early 1960s by McCann, Taleisnik, and Friedman (144) and Campbell, Feuer, and Harris (see 90). Approximately 15 years later, groups headed by Guillemin (86) and Schally (199) established the amino acid sequence of the mammalian decapeptide GnRH (mGnRH). Recently, a second molecular form of GnRH has been identified in brains of several eutharian mammals, including humans (232), tree (113) and musk (54) shrews, and golden moles (118). This second molecular form has been identified as chicken II GnRH (cGnRH-II), which has also been found in many other nonmammalian vertebrate species (118, 159). Using immunocytochemical procedures, the neural distribution of GnRH producing neurons has been specified in numerous vertebrate species. Mammalian GnRH and cGnRH-II producing neurons are concentrated in two separate anatomical locations. Neurons producing the mammalian form of GnRH are distributed in a loose rostral–caudal continuum in the ventral forebrain. There are clear species (10, 181, 227) differences in the distribution of these GnRH neurons (Fig. 3); however, these differences are not correlated with individual species' status as spontaneous as opposed to induced ovulators. The principal projection of mGnRH neurons is to the median eminence, where terminals of these neurons have been observed in the pericapillary space of the hypophyseal portal plexus (78, 205). The mammalian form of GnRH is released from the median eminence in a pulsatile fashion (e.g., 30, 68). Neurons expressing cGnRH-II reside in the midbrain (54, 113, 118, 232). The major terminal field for these cGnRH-II neurons is the medial habenula, and in contrast to forebrain mGnRH fibers, few cGnRH-II fibers are visible in the median eminence (54). The fact that cGnRH-II has been conserved in species that have diverged evolutionarily suggests that it may have an important function. Several studies (76, 129, 147, 253) have shown that cGnRH-II is effective in releasing LH and FSH from the mammalian pituitary. However, mGnRH is a much more potent stimulator of pituitary gonadotropin secretion (76, 147). Also, in contrast to mGNRH, intracerebroventricular infusion of cGnRH-II had no effect on female sexual behavior in musk shrews (200). More research is needed to specify the function of this second form of GnRH in the mammalian brain. Henceforth in this review we will only discuss the neuroendocrine mechanisms controlling synthesis and release of mammalian GnRH in the forebrain of induced ovulators.

GnRH Secretion

The introduction of push–pull perfusion (PPP) and microdialysis techniques to measure *in vivo* release of hypothalamic neuropeptides in conscious, freely moving animals enabled investigators to measure GnRH secretion into the hypophyseal portal system. To date, the rabbit is the only induced ovulating species in which these techniques have been used. Groups headed by Ramirez (177) and Spies (169) showed independently that the *in vivo* release of GnRH is pulsatile in the rabbit. The pulsatile pattern of tonic GnRH release in the
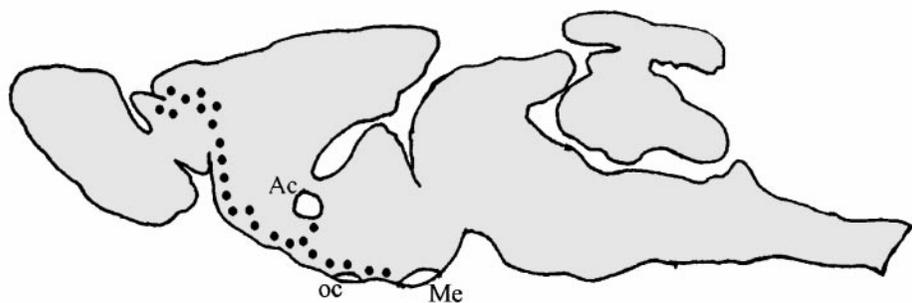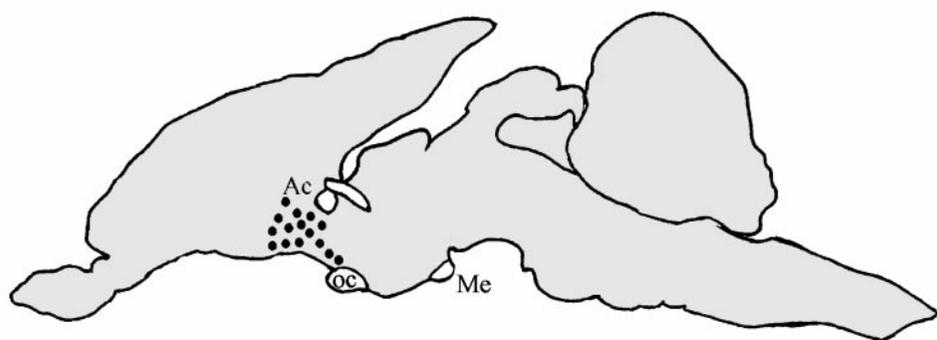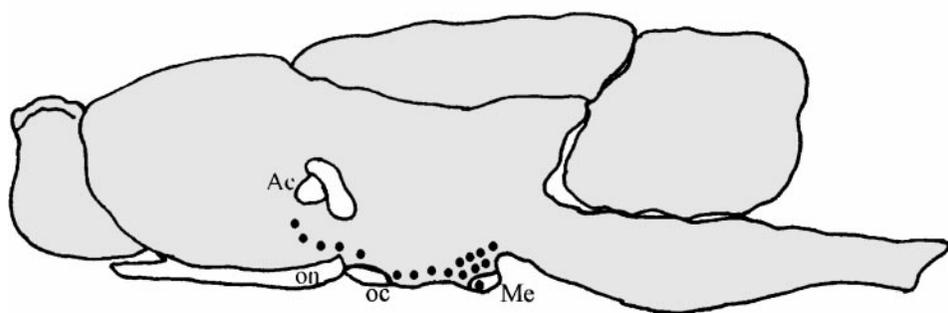
FIG. 3. The distribution of neurons (●) immunoreactive for mammalian GnRH in sagittal views of the brains of the musk shrew, rabbit, and ferret. The number of dots does not represent the actual number of GnRH neurons present in each species. The brains are not drawn to scale. Ac, anterior commissure; oc, optic chiasm; on, optic nerve; Me, median eminence.

rabbit resembles that seen in spontaneous ovulators such as the rat (131), the monkey (132), and the sheep (130). Both research groups (114, 136) have also shown that the receipt of intromissions induced a rapid increase in hypotha-
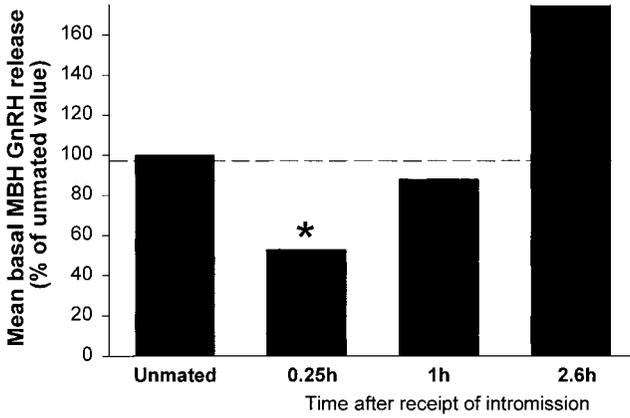
Adapted from Lambert et al. (Physiol Behav 1992; 52: 809-813)

FIG. 4. Mean basal *in vitro* release of GnRH was significantly decreased in mediobasal hypothalamic tissue of estrous female ferrets killed 0.25 h after the onset of intromission compared to unmated estrous females. The *in vitro* release of GnRH had recovered in females killed 1 or 2.6 h after the onset of intromission. Data are expressed as percentage of unmated value. *$P < 0.05$ compared to unmated value.

lamic GnRH release. The magnitude of this mating-induced output of GnRH was far greater (~50–100-fold) than what has been reported for other species, including rats (191) and sheep (43), on proestrus.

In ferrets (121), mating-induced changes in GnRH release from hypothalamic slices were assessed by *in vitro* perifusion. Perifusion chambers contained individual slices from the MBH which were collected from female ferrets that had been either mated or left unpaired. The *in vitro* release as well as the content of GnRH in MBH slices were significantly lower in estrous females killed 0.25 h after receipt of an intromission than in unmated estrous females and in estrous females killed 1 or 2.6 h after the onset of mating (121; Fig. 4). This suggests that the receipt of an intromission induces a release of GnRH from the female's MBH which quickly depletes neuronal terminals of the peptide. Additional, independent evidence (19) of an immediate release of GnRH following mating in the ferret is that fewer GnRH-immunoreactive (IR) perikarya were detected in the MBH of ovariectomized, estradiol-primed female ferrets killed 20 min after receiving mechanical vaginal–cervical stimulation. In the vole (218), a similar drop in GnRH content was found in dissected hypothalami of females killed 5 min after mating. Taken together, these results suggest that the mating-induced preovulatory LH surge is stimulated by an immediate release of GnRH from the MBH in rabbit, vole, and ferret. In contrast to these induced ovulating species, a single mating bout, which typically includes about 20 mounts with and without intromission terminating in 1 ejaculation, initially increased GnRH-IR cell numbers and content in the POA and other forebrain areas of female musk shrews (55). Within 24–40 h after receipt of an ejaculation, females that ovulated had fewer GnRH-IR

neurons and lower GnRH content in the entire brain than females that did not ovulate. Unfortunately, the exact time of the preovulatory LH surge could not be determined since there is no shrew LH assay available (55). However, the drop in forebrain GnRH content presumably reflects its release from the median eminence. These results imply that the female musk shrew represents a special case of induced ovulation in which receipt of intromissive stimulation does not lead to a rapid release of GnRH and an immediate preovulatory surge in LH.

Neuronal Fos/GnRH Colocalization

The expression of immediate early genes, such as *c-fos* and *c-jun,* has been used as a marker of neural activation in many neuroendocrine systems, including GnRH neurons (99). Dual-label immunocytochemical visualization of Fos and GnRH protein has been used in both ferrets (122, 227) and rabbits (28) to specify the population of GnRH neurons which is activated by mating-associated stimuli leading to a preovulatory LH surge. In the ferret (122), receipt of an intromission significantly increased the percentage of GnRH neurons expressing Fos protein in the MBH of estrous females killed 1 h after the onset of a 5-min intromission. However, the percentage of Fos- or Jun-expressing GnRH neurons returned to basal levels 3 h after onset of intromission (227; Fig. 5), even though the preovulatory LH surge lasts for at least 12 h in this species (33). It therefore seems unlikely that immediate early gene products such as Fos or Jun that are formed in GnRH neurons play a critical role in the termination of the LH surge since they disappear long before the surge is completed. Fos and Jun are also probably not involved in the initiation of the preovulatory LH surge since the percentage of Fos-IR GnRH neurons did not rise until 1–1.5 h after mating (227; Fig. 5). This is long after the initial release of GnRH, which occurs within 15 min of mating (121). It is possible that Fos, perhaps by increasing GnRH gene transcription (60, 99), plays a role in replenishing intracellular MBH GnRH stores with the decapeptide within the first hour after the onset of intromission (121).

At the time of the steroid-induced preovulatory LH surge, a similar induction of Fos protein within GnRH neurons has been observed in spontaneously ovulating species, including mice (245), rats (125), hamsters (17, 60), and sheep (155). Interestingly, artificial vaginal–cervical stimulation was successful in inducing Fos protein in GnRH neurons of the female rat even in the absence of circulating steroids (172). Rajendren *et al.* (176) demonstrated that repeated mating induced Fos protein in GnRH neurons of ovariectomized, estradiol-treated female rats. Moreover, Wu *et al.* (245) reported that mating augmented the percentage of Fos-IR GnRH neurons induced by the administration of estradiol and progesterone and extended this period of enhanced colocalization in female mice. These results suggest that the neural pathways by which sensory stimuli associated with mating reach and activate forebrain GnRH neurons in induced ovulators are also present in the rat and mouse.
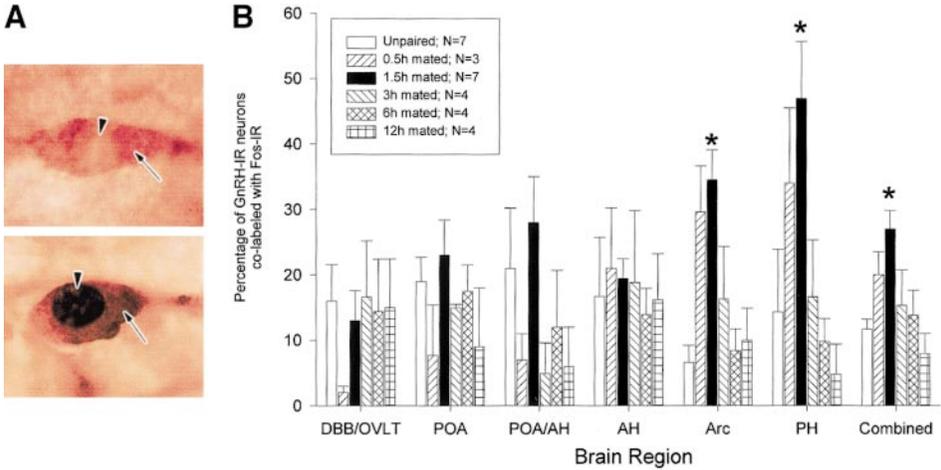
FIG. 5. Activation of mediobasal hypothalamic GnRH neurons by mating-associated stimuli in estrous female ferrets. (A) Photomicrograph showing a single-labeled (top) and a double-labeled (bottom) GnRH-immunoreactive (IR) neuron with Fos protein in the nucleus. Arrows indicate GnRH-IR cytoplasm; arrowheads indicate the nucleus. (B) Mean percentage ($\pm$ SEM) of GnRH-IR neurons colabeled with Fos-IR at the level of the diagonal band of broca/organum vasculosum of the lamina terminalis (DBB/OVLT), the preoptic area (POA), the border of the preoptic area/anterior hypothalamus (POA/AH), the anterior hypothalamus (AH), the arcuate nucleus (Arc), the posterior hypothalamus (PH), and all these brain regions combined. Groups included unpaired and mated estrous female ferrets killed 0.5, 1.5, 3.0, 6.0, or 12.0 h after receipt of one 5-min intromission. *$P < 0.05$ compared to the unpaired group. Adapted from 227.

GnRH Biosynthesis

There is increasing evidence that the amount of GnRH peptide available for secretion into the portal circulation is regulated by a combination of transcriptional and posttranscriptional events. These include the rate of transcription of the proGnRH gene, polyadenylation and 5′ capping of the resulting RNA, processing of the GnRH primary transcript into mRNA, transport of the mRNA to the cytoplasm, translation rate of the mRNA into pre-pro-GnRH peptide, and processing of the precursor into GnRH decapeptide (83). To date the ferret (8) is the only induced ovulator in which MBH GnRH mRNA levels have been assessed in relation to mating-induced preovulatory LH surges. The receipt of intromissive stimulation by estrous ferrets stimulated GnRH release from the MBH that initially depleted stores of the peptide (121; Fig. 4). However, MBH GnRH stores were replenished within 1 h after the onset of intromission, suggesting that the preovulatory GnRH surge in the ferret may be associated with an increase in GnRH biosynthesis. Bakker *et al.* (8) compared MBH GnRH mRNA levels among groups of estrous ferrets killed at different times during the mating-induced preovulatory LH surge. GnRH mRNA levels in the MBH were assessed by means of *in situ* hybridization using a ^{35}S-labeled synthetic oligoprobe complementary to the GnRH coding region (bases 102–149) of the human cDNA (2). No evidence of increased MBH
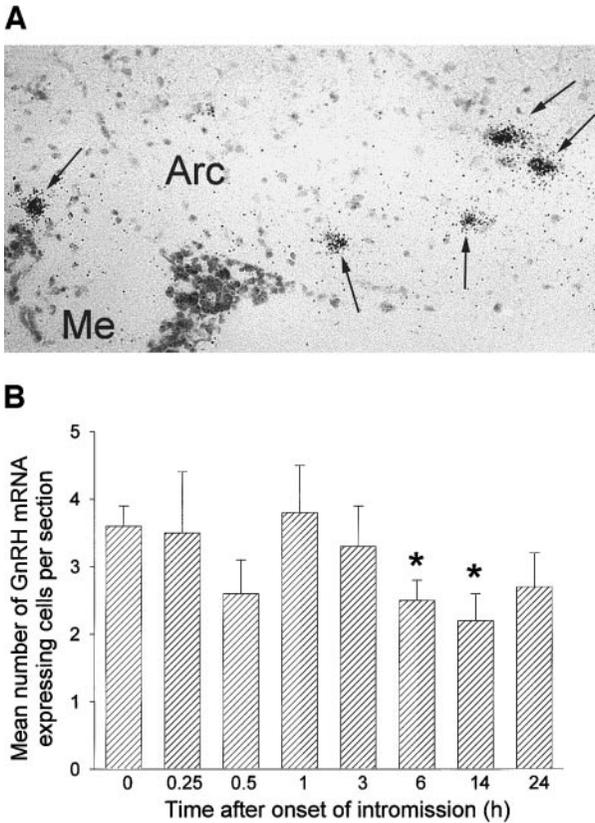
FIG. 6. Effect of receipt of an intromission on mediobasal hypothalamic GnRH mRNA levels in estrous female ferrets. (A) Photomicrograph of cells expressing GnRH mRNA in the arcuate region (Arc) and the median eminence (Me) of an unmated estrous female ferret. Arrows indicate labeled cells. (B) Mean ($\pm$ SEM) number of GnRH mRNA-expressing cells in the arcuate region of estrous female ferrets. $*P < 0.05$ compared with unmated (0-h) estrous females. Adapted from 8.

GnRH mRNA levels was found during the ferret's mating-induced preovulatory LH surge (8; Fig. 6). Instead, the number of GnRH mRNA-containing cells dropped significantly in the arcuate region beginning 6 h after onset of intromission and remained low thereafter. This decrease probably reflected a reduction in cellular GnRH mRNA content so that cells expressing decreased levels of mRNA were no longer detected with the *in situ* hybridization method used. Indeed, cellular levels of GnRH mRNA in the arcuate region decreased significantly in estrous females killed 14 h after the onset of intromission.

The absence of any increases in GnRH mRNA levels during the mating-induced preovulatory LH surge suggests that posttranscriptional events, such as increased translation of GnRH mRNA, increased conversion of precursor into mature GnRH peptide, and/or increased transport of GnRH peptide to the nerve terminals, contribute to the previously (121) observed postcoital replenishment of GnRH stores in the ferret's MBH. There is additional, independent

evidence (19) that MBH GnRH biosynthesis is regulated by posttranscriptional mechanisms in the ferret. The number of protein-synthesizing organelles, particularly Golgi complexes, was significantly increased in GnRH neurons of female ferrets that received vaginal–cervical stimulation.

Numerous studies (reviewed in 83, 190) have been conducted in spontaneously ovulating species, including rats and sheep, to determine whether the steroid-induced preovulatory release of GnRH depends, in part, on increased GnRH gene transcription in POA neurons. Unfortunately, results obtained in these studies have been inconclusive. In the rat, cellular GnRH mRNA levels and/or GnRH mRNA cell numbers have been reported to increase in the organum vasculosum of the lamina terminalis (OVLT)/rostral POA either around the time of (164, 252) or preceding (82, 174) the preovulatory LH surge. By contrast, in other studies (74, 139) no changes in POA GnRH mRNA levels were seen across the rat's estrous cycle. In the ewe (58, 91) GnRH mRNA levels have been reported to decrease in the POA at the time of the preovulatory LH surge. The finding in ferrets (8) that the mating-induced preovulatory LH surge is accompanied by progressively decreasing levels of MBH GnRH mRNA is comparable to those earlier findings in the sheep (58, 91). However, the temporal relationship between the changes observed in POA or MBH GnRH mRNA levels and LH release differs between ferrets and sheep. In the ferret (8), MBH GnRH mRNA levels started to decrease during the peak phase of the LH surge and remained low until its end, whereas in the sheep (91) GnRH mRNA levels decreased in the POA before the onset of the LH surge. This difference points once again to the existence of different neuroendocrine mechanisms controlling the preovulatory release of GnRH, and consequently LH, in induced and spontaneously ovulating species.

Sex Differences in GnRH Secretion

The ability to generate preovulatory LH surges is sexually dimorphic in induced ovulators as in numerous spontaneously ovulating species (Fig. 7). Mating induced LH surges in female, but not in male, voles (84), ferrets (32), and rabbits (105, 247). In male ferrets (32) LH secretion was actually reduced significantly after an intromission was achieved with a female. Such sex dimorphisms in mating-induced (and spontaneous) LH secretion probably originate from sex differences in the neural inputs to GnRH neurons since the anterior pituitary is not sexually differentiated (12, 32). Gonadectomized male and female ferrets (32) showed similar LH responses to exogenous GnRH treatment, regardless of whether they were tested with or without concurrent estradiol treatment. More recently, Yang *et al.* (247) provided direct evidence for the absence of a coitus-induced surge in MBH release of GnRH as well as in plasma LH in the male rabbit.

Among spontaneously ovulating species, sex dimorphisms in the occurrence of preovulatory LH secretion have been studied most extensively in the laboratory rat (89, 161, 173). A preovulatory LH surge could be induced in female,
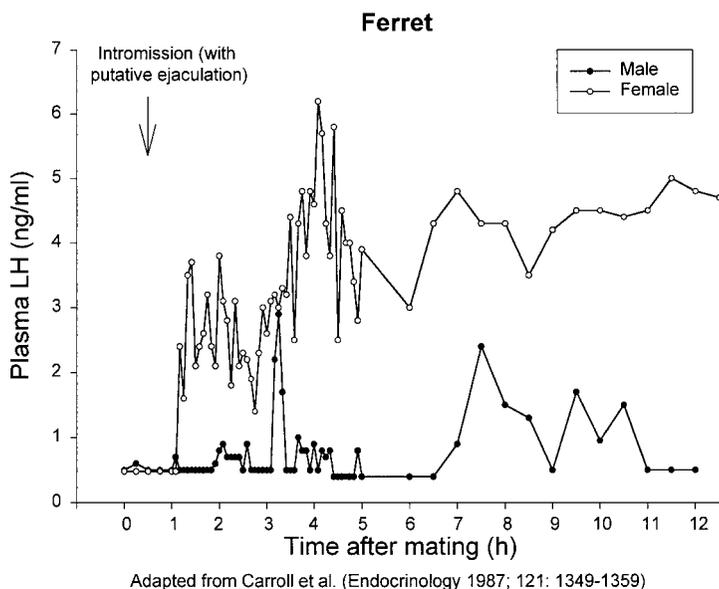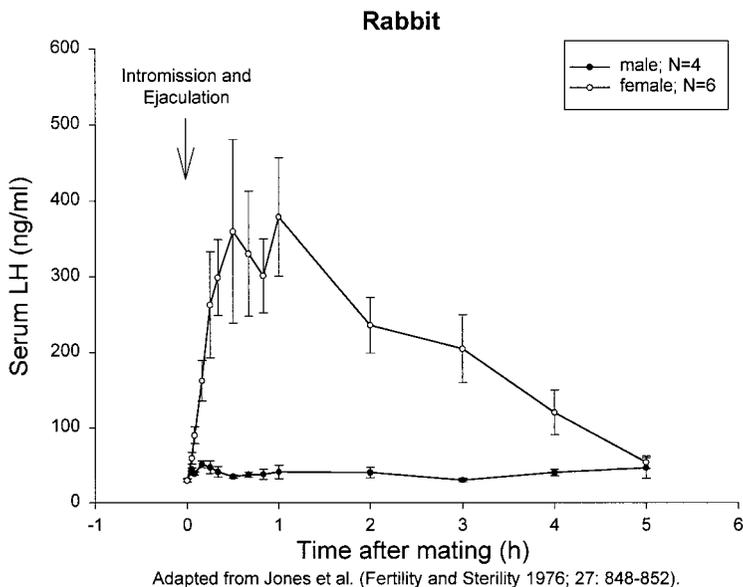
Rabbit

Adapted from Jones et al. (Fertility and Sterility 1976; 27: 848-852).

Ferret

Adapted from Carroll et al. (Endocrinology 1987; 121: 1349-1359)

FIG. 7. Sexual dimorphism in mating-induced LH secretion in the rabbit and ferret. For the rabbit, data represent means ± SEM; for the ferret, individual profiles of LH release following mating in a representative male and female tested while in breeding condition.

but not in male, rats following gonadectomy and administration of estradiol (161). The sexual differentiation of the GnRH–LH surge system depends on the perinatal actions of testosterone and/or its estrogenic metabolite on the

male rat's hypothalamus (161, 220, 226). However, the site and mechanism by which testosterone acts to masculinize the neuroendocrine circuitry controlling GnRH release in males is unclear. Using both immunocytochemical (122) and *in situ* hybridization procedures (7), no sex differences were found in the number, anatomical distribution, or morphology of GnRH neurons in the ferret, as in numerous spontaneously ovulating species (241, 242, 243). By contrast, in the musk shrew (184), males had significantly more GnRH-IR (of both mammalian and chicken-II forms) cells in the forebrain (medial septum, diagonal band of broca, POA, hypothalamus) and midbrain than females. Ovariectomy eliminated this sex difference, suggesting that sex differences in circulating gonadal steroids in adulthood are responsible for this sex dimorphism. As such, these variations in GnRH cell numbers do not represent actual sex difference in the total number of GnRH-producing neurons in the musk shrew brain (184). Thus the sex dimorphism in the ability of mating or ovarian steroids to stimulate a preovulatory LH surge does not appear to result from sex differences in the number or distribution of GnRH neurons per se. Instead, this sex dimorphism probably reflects differences in the input circuits to forebrain GnRH neurons. Indeed, mating with intromission induced nuclear Fos-IR in MBH GnRH neurons in estrous female, but not male ferrets (122, 228) and rabbits (28). The distribution of mating-induced Fos-IR in non-GnRH forebrain neurons was also sexually dimorphic in the ferret: Fos expression was significantly higher in the POA, bed nucleus of the stria terminalis (BNST), and ventrolateral hypothalamus (VLH) of estrous females compared to breeding males (228). These results point to sex differences in the processing of sensory inputs to the ferret's MBH, including the GnRH neurons located in this region.

REGULATION OF GnRH SECRETION AND BIOSYNTHESIS BY OVARIAN STEROID HORMONES

As explained earlier, estradiol but not progesterone is required for the induction of behavioral receptivity in induced ovulators (e.g., 13, 102). However, it is unclear to what extent estradiol and/or progesterone act directly on the neural mechanisms controlling GnRH biosynthesis and release leading to a mating-induced preovulatory LH surge. Presumably, there is no direct action of estradiol or progesterone on GnRH neurons in induced ovulators (222) since these neurons lack nuclear estrogen or progesterone receptors (95, 126, 203, 210, 224). There is recent evidence (206) of estrogen receptor α and β mRNA expression by POA GnRH neurons that clearly fluctuated over the rat's estrous cycle, suggesting a possible direct action of estradiol on GnRH function. However, many researchers have looked for estrogen receptor protein in GnRH neurons without success (e.g., 95, 126, 203, 210, 224). Until more evidence is forthcoming, we are going to presume that GnRH neurons do not express estrogen receptors.

Induced ovulators generally do not show spontaneous LH surges during their reproductive cycles, suggesting that the positive feedback actions of steroid hormones on GnRH release are reduced or absent in these animals. Likewise, there is little indication that administration of estradiol with or without progesterone induces preovulatory LH secretion in induced ovulators (e.g., 12, 13, 102, 149, 150, 197). In addition, there is no clear evidence that progesterone has any positive feedback actions on LH release in induced ovulators. In rabbits (96) progesterone and 20α-hydroxyprogesterone (20α-OHP) levels increase significantly in the course of the preovulatory LH surge, whereas in ferrets (219), voles (36), and cats (236) progesterone levels do not start to rise significantly until 3–5 days after mating. Whether progesterone or 20α-OHP facilitates the postcoital release of LH in the rabbit is still unclear (80, 97, 179). Infusion of pulses of progesterone directly into the hypothalamus stimulated the release of GnRH in young, intact female rabbits (177). However, no such effect was observed when progesterone was infused continuously (134). Likewise, pulses of 20α-OHP infused directly in the hypothalamus stimulated the release of GnRH in young, intact rabbits (135). This result supports the hypothesis of Hilliard *et al.* (97) that 20α-OHP exerts positive feedback effects on the hypothalamus and pituitary by prolonging the post-coital output of LH from the pituitary. However, Goodman and Neill (80) did not observe any contribution of 20α-OHP to LH release. In fact, female rabbits that were ovariectomized within 15 min after receipt of an ejaculation showed the same pattern of LH release as intact rabbits, suggesting that the ovaries do not contribute to the preovulatory LH surge. Furthermore, subcutaneous administration of 20α-OHP by silastic capsules actually attenuated MBH GnRH release without affecting LH or FSH release in ovariectomized female rabbits treated concurrently with either estradiol or no hormone (169).

A monosynaptic pathway between the suprachiasmatic nuclei (SCN), the circadian pacemaker, and GnRH neurons located in the rat's OVLT/POA is likely part of the neural circuit which mediates the stimulatory actions of ovarian steroids on GnRH release (217). In rats, the preovulatory LH surge occurs at a fixed circadian time that is synchronized by the prevailing light–dark cycle. For instance, in female rats (reviewed in 108) maintained on 14-h light and 10-h darkness (lights on between 05:00 and 19:00 h) the preovulatory LH surge typically occurs on the day of proestrus between 14:00 and 16:00 h. It is unclear to what extent this timing of the LH surge to the light/dark cycle can be applied to other spontaneously ovulating species. In the rhesus monkey (112), for example, the estradiol-induced preovulatory LH surge does not appear to be coupled to the light/dark cycle. By contrast, in humans (65), the preovulatory LH surge usually occurs in the morning around 9:00 h. Destruction of the SCN (5, 27) or exposure to constant illumination (225) leads to a cessation of ovarian cyclicity and induces a state of persistent estrus in female rats. These animals will show coitus- but not steroid-induced ovulation. Thus, a spontaneous ovulator, such as the rat, can be transformed into an induced ovulator by ablation of the SCN–GnRH neural pathway. This finding raises the possibility that the SCN–GnRH pathway is either absent or weakly orga-
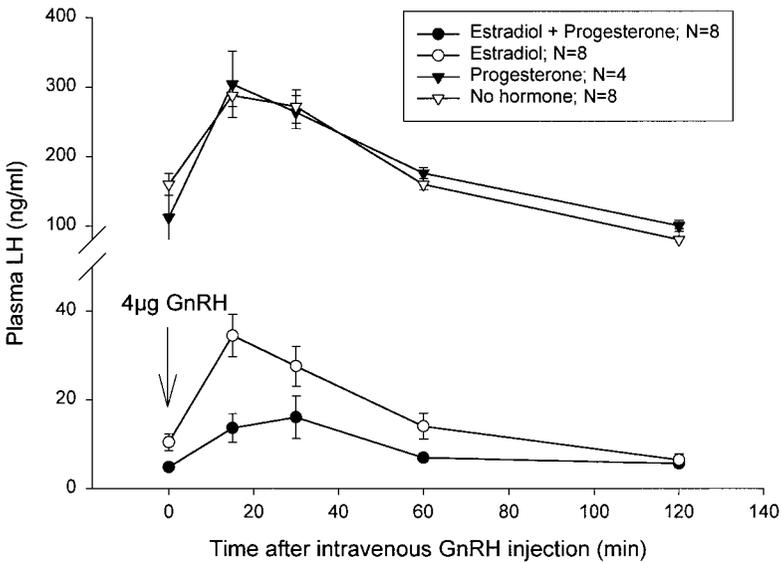
FIG. 8. Effect of chronic exposure of ovariectomized female ferrets to subcutaneous silastic capsules containing progesterone, estradiol, or no hormone on the stimulation of LH secretion by intravenous injection of GnRH. Data are represented as means ± SEM.

nized in induced ovulators. In fact, ferrets show very weak circadian rhythms in locomotor activity levels (209) and in the display of maternal behavior (11).

Numerous studies (31, 106, 156, 169) suggest that ovarian steroids exert primarily negative feedback actions on hypothalamic GnRH release and pituitary LH secretion in induced ovulators. Removal of the ovaries caused a rapid increase in pituitary LH secretion in rabbits (169), cats (106), ferrets (31), and voles (156). Conversely, administration of estradiol following ovariectomy suppressed plasma LH levels to those found in intact rabbits (169), cats (106), and ferrets (31). The few data (121, 169) that are available on GnRH release after gonadectomy point to species differences among induced ovulators, as have been reported for spontaneously ovulating species (29, 110, 111). In the female rabbit (169), ovariectomy enhanced pulsatile release of GnRH in the arcuate median eminence as measured by push–pull perfusion. By contrast, ovariectomy of estrous female ferrets (121) caused a decrease in the *in vitro* release and content of GnRH peptide in the MBH.

There is no indication that progesterone exerts negative feedback effects on LH release in induced ovulators. Plasma progesterone levels are very low in both unmated estrous rabbits (179) and ferrets (219). Furthermore, ovariectomized female ferrets which received either progesterone or no hormone had similar plasma LH levels which were significantly higher than those of ovariectomized, estrogen-primed female ferrets (13). In the ferret, the ability of a single intravenous injection of GnRH to stimulate LH release was significantly lower in ovariectomized female ferrets implanted subcutaneously with silastic

capsules containing estradiol and progesterone than in ferrets given only estradiol (Fig. 8; 13). Administration of progesterone alone to ovariectomized females did not affect the ability of exogenous GnRH to stimulate LH release. The finding that the inhibitory action of progesterone on pituitary responsiveness to GnRH depends on concomitant exposure to estradiol is correlated with the finding that estradiol doubled cytosolic progesterone receptor concentrations in the pituitary gland of ovariectomized ferrets (13). Donovan *et al.* (62) reported that plasma concentrations of progesterone dropped significantly in anestrous ferrets shortly after they were switched to a long-day photoperiod. Progesterone levels remained low during the subsequent period of 4–5 weeks prior to the onset of estrus. Ryan (188) observed episodic bursts of LH secretion during this same period prior to first estrus onset in female ferrets. These results raise the possibility that a reduction in the feedback effect of progesterone on GnRH neurons or pituitary gonadotropes contributes to the onset of estrus in this induced ovulator.

GnRH Biosynthesis

The *in vitro* release as well as the content of GnRH in MBH slices were reduced by ovariectomy of female but were not affected by castration of breeding male ferrets (121). By contrast, the time course of the rise in pulsatile LH secretion following estradiol withdrawal was very similar in gonadectomized male and female ferrets (31). These latter findings suggest that gonadal steroids exert strong negative feedback actions on LH secretion in ferrets of both sexes. However, GnRH release and perhaps also GnRH biosynthesis may be differentially regulated by gonadal steroids in male and female ferrets. It was therefore of interest to compare the effects of gonadectomy and steroid replacement on MBH GnRH mRNA cell number and content in ferrets of both sexes (7). Isotopic *in situ* hybridization showed that the number of MBH neurons and the cellular content of GnRH mRNA were equivalent among groups of gonadally intact ferrets in breeding condition as well as in gonadectomized steroid-treated and gonadectomized oil vehicle-injected ferrets of both sexes (7). This finding that castration of male ferrets had no effect on MBH GnRH mRNA cell number agrees with an earlier study by Tang *et al.* (211). Thus, the postgonadectomy rise in plasma LH levels, which is equivalent in the two sexes, does not depend on increased MBH GnRH gene transcription leading to increased GnRH mRNA levels. In addition, gonadal steroid hormones do not appear to modulate MBH GnRH mRNA levels in the ferret (7, 211). These data agree with results for the rat (66, 115, 139, 235), which also failed to detect any significant changes in hypothalamic GnRH mRNA levels after steroidal manipulations. By contrast, there are also numerous reports (reviewed in 83, 190) showing either increases or decreases in POA GnRH mRNA levels after gonadectomy in rats, although these changes in GnRH mRNA levels are relatively small compared to the amount of LH released following gonadectomy. Thus, the postgonadectomy rise in LH does not seem to

depend on dramatic increases in hypothalamic GnRH gene transcription in either rats or ferrets. Instead, increased LH secretion following gonadectomy may result from an increase in the sensitivity of pituitary gonadotropes to lower amplitude GnRH pulses from the median eminence (110, 121). By contrast, in the rabbit (169), the postgonadectomy rise in LH is associated with an enhanced secretion of GnRH from MBH nerve terminals. This increase in GnRH release could be the result of increases in GnRH gene transcription and/or GnRH mRNA translation, suggesting that different neuroendocrine mechanisms underlie the postgonadectomy rise in LH secretion in rabbits as opposed to ferrets.

NEUROTRANSMITTERS AND NEUROPEPTIDES INVOLVED IN THE PREOVULATORY ACTIVATION OF GnRH NEURONS

The neural pathways via which sensory stimuli associated with mating reach and activate forebrain GnRH neurons in induced ovulators are still poorly understood. GnRH neurons lack estrogen as well as progesterone receptors in both spontaneous and induced ovulators, implying that these steroid hormones act on GnRH neurons via interneurons (reviewed in 94). Therefore, much of the work within the field of GnRH neurobiology has centered on components of the neural circuits that convey steroidal signals and/or sensory inputs to forebrain GnRH neurons. Pharmacological manipulation of various neurotransmitter and peptidergic systems has been shown to affect GnRH and subsequent LH release in female mammals. Most of this work has been done in spontaneous ovulators, including rats (9, 25, 93, 133, 153) and monkeys (reviewed in 208). To date, the rabbit (reviewed in 179, 208) and ferret (120, 229) are the only induced ovulating species in which pharmacological studies have been conducted to analyze the neuroendocrine signals which control the postcoital release of GnRH from the MBH. Norepinephrine, neuropeptide Y, and opioid peptides are the best studied neurotransmitter/neuropeptides in this context.

Norepinephrine

Much evidence suggests that the catecholamine norepinephrine (NE) facilitates GnRH release in the estrous female rabbit's MBH: (1) intravenous administration of dibenamine, an α-adrenergic antagonist blocked coitus-induced ovulation (198); (2) intracerebroventricular administration of NE stimulated GnRH secretion (170, 178, 195); (3) mating induced an immediate and substantial increase in MBH NE several minutes prior to the initiation of the preovulatory GnRH surge (Fig. 9; 247), whereas prazosin, an α_1-adrenergic receptor blocker administered either in the arcuate region median eminence or into the third ventricle, attenuated the mating-induced GnRH surge (246); and (5) desipramine (DMI), a specific NE transporter (NET) protein blocker ad-

Mated Female Rabbits
(N=4)
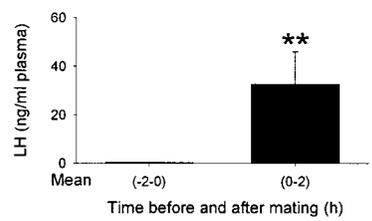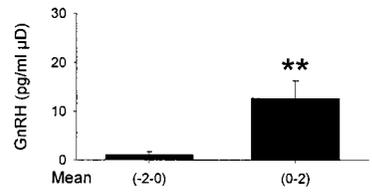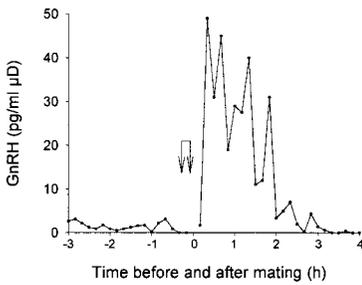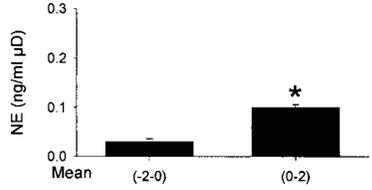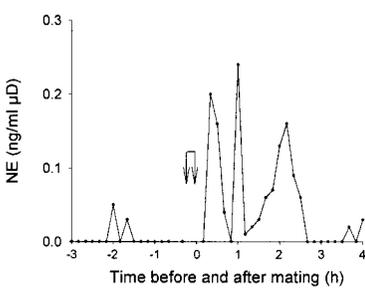
Mated Male Rabbits
(N=6)
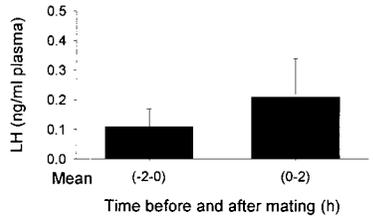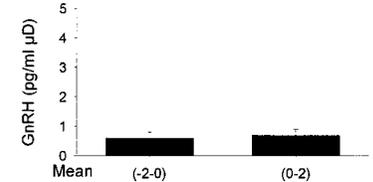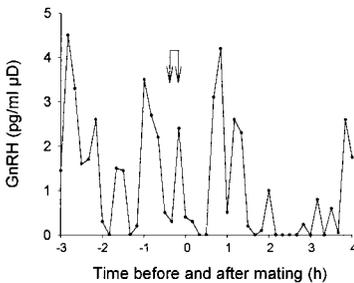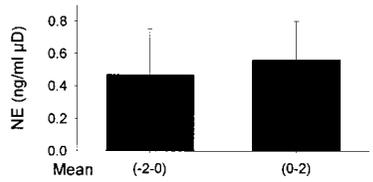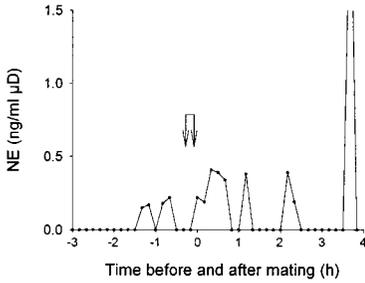
Adapted from Yang et al. (Endocrinology 1996; 137: 2683-2693)

ministered into the arcuate region median eminence elevated GnRH release by increasing local NE concentrations (167).

Noradrenergic cells in the rabbit midbrain have been analyzed (168). Cells containing NE, NET, and/or tyrosine hydroxylase (TH), the rate-limiting enzyme in NE synthesis, were localized in the rabbit brain stem using combined immunocytochemistry for dopamine-β-hydroxylase (DBH), the enzyme specific for NE synthesis, and *in situ* hybridization for NET and TH (168). NE-, NET-, and TH-containing cells were localized in the A1 and A2 cell groups and the area postrema of the medulla of the female rabbit. The pons, the locus coeruleus (LC or A6 cell group), and the A5 cell group all expressed NE, NET, and TH. The distribution of NE cells in the rabbit is similar to that of the rat (e.g., 100). Yang *et al.* (248) showed that the receipt of mating stimulation increased TH and NET mRNA levels in the LC of female rabbits. Likewise, in estrous ferrets (229), the receipt of an intromission significantly augmented the percentage of TH-IR neurons colabeled with nuclear Fos protein in both the LC (A6) and the rostral A2 cell groups. Taken together, these data demonstrate a pivotal role for NE in the preovulatory release of GnRH in rabbits and ferrets. The available evidence points to the LC as one of the brain sites important for conveying genital–somatosensory inputs to GnRH neurons. Lesions of the LC decreased NE input into the medial POA and MBH and consequently blocked the preovulatory LH surge in intact cycling female rats (4). Conversely, electrical stimulation of the LC significantly augmented LH secretion in the rat that was otherwise induced by electrochemical stimulation of the medial POA. Only the brainstem noradrenergic neurons of the A1 and A2 cell groups project to the immediate vicinity of the GnRH cell bodies located in the rat's rostral preoptic area (244), suggesting that LC neurons constitute one segment of a circuit mediating the stimulatory effect of ovarian steroids on MBH GnRH output. No information is available about the projections of LC NE neurons in either rabbits or ferrets. Perhaps in induced ovulators genital–somatosensory inputs generated by receipt of an intromission interact with steroidal signals to activate the same NE pathways that in spontaneous ovulators are activated by ovarian steroids alone. An increased release of GnRH from the MBH neurons results in both cases.

The activation of the noradrenergic system by mating stimuli is sexually dimorphic in rabbits (247) and ferrets (229). Male rabbits did not display surges in MBH levels of NE or GnRH after coitus (Fig. 9; 247). Correspondingly, TH and NET mRNA levels were not enhanced in the LC of male rabbits

FIG. 9. Sexual dimorphism in the hypothalamic secretion of GnRH and norepinephrine (NE) after mating in rabbits. Levels are shown of NE and GnRH in 10-min microdialysate and of LH in plasma samples from mated males and females. The line plots show individual profiles of hypothalamic NE and GnRH in representative males and females. Arrows indicate the interval when mating occurred. The bar plots show mean ($\pm$ SEM) levels of NE, GnRH, and LH before ($-2-0$) and after ($0-2$) mating. Note that due to high levels of GnRH in mated females, different scales were used to plot the patterns of GnRH in mated males and females.

after mating (248). Likewise in male ferrets (229), mating with intromission did not significantly increase the percentage of TH-IR neurons colabeled with Fos-IR in any brain stem region. These results point to a sexually dimorphic processing of coital signals in the brain stem that results in the activation of brainstem noradrenergic neurons only in females.

Neuropeptide Y

The effects of neuropeptide Y (NPY) on MBH GnRH secretion are well documented in the rabbit. NPY is released from the hypothalamus in a pulsatile manner (117). Intrahypothalamic infusion of NPY stimulates GnRH release in ovariectomized, estradiol-treated female rabbits whereas it inhibits GnRH pulsatile release in ovariectomized does (117, 166). These effects of NPY are similar to those of NE (170, 178). The mechanism by which NPY stimulates GnRH secretion is currently unknown. However, there is evidence that NPY stimulates GnRH release via an α-adrenergic pathway. When ovariectomized, estrogen-treated female rabbits were infused with prazosin, an α-1 receptor antagonist, NPY did not stimulate GnRH release (16). However, administration of yohimbine, an α-2 receptor antagonist, did not block the stimulatory action of NPY on GnRH release. These data suggest that NE is an intermediary between NPY and GnRH neurons. NPY mRNA-containing neurons have been localized in the lateral tegmentum (A1) and the nucleus of the solitary tract (A2), the same regions that were immunoreactive for DBH, in the rabbit (168). In the rat (52, 193), the A2 cell group projects to the paraventricular and supraoptic nuclei of the hypothalamus. However, this pathway probably does not contain NPY as the putative transmitter in the rat (194). It is possible, however, that in the rabbit this pathway involves NPY.

Opioid Peptides

The role of endogenous opioid peptides in the regulation of GnRH secretion has been studied extensively (reviewed in 72, 109, 249). There is considerable evidence that endogenous opioid peptides tonically inhibit POA GnRH neurons in rats. Chronic treatment with morphine blocked GnRH secretion (42) and decreased POA GnRH mRNA levels (133), whereas the opioid receptor antagonist naloxone increased plasma LH levels and POA GnRH mRNA levels. Several studies suggest that endogenous opioids inhibit LH secretion in rabbits (163, 250), ferrets (120), and cats (101). Female rabbits showed a dramatic rise in LH pulse amplitudes and mean LH levels when given an intravenous infusion of naloxone 2 weeks after gonadectomy. However, no LH response to naloxone was obtained in female rabbits that were gonadally intact and in estrus (163). In another study (250), naloxone effectively raised LH pulse amplitudes in female rabbits tested after ovariectomy. Treatment with estradiol blocked this LH response to naloxone. Likewise, administration of nalox-

one increased LH pulse frequency in both gonadectomized male and female ferrets (120). As in rabbits, these stimulatory effects of naloxone on LH secretion disappeared when estradiol was given to the gonadectomized ferrets. In cats (101), intravenous injections of naloxone induced LH release in ovariectomized females that were anesthetized; however, the effects of estradiol replacement on this LH response to naloxone were not studied.

In spontaneous ovulators it has been suggested that the neural mechanisms underlying the steroid-induced preovulatory surge of GnRH and LH secretion may include both excitation and the removal of inhibitory inputs (108). This disinhibition may reflect, in part, a reduction in opioid inhibitory tone. By contrast, in induced ovulators the neural signals that induce a preovulatory LH surge may be primarily excitatory. As such, there is no important role for endogenous opioid peptides in the regulation of MBH GnRH release in these animals. In rats, arcuate nucleus concentrations of β-endorphin (e.g., 192) and proopiomelanocortin (POMC; 24) mRNA levels, the precursor of β-endorphin, have been found to fluctuate over the estrous cycle. POMC mRNA levels declined before the onset of the preovulatory LH surge and then rose again dramatically during the surge (24). Estrogen treatment of ovariectomized female rats decreased arcuate levels of POMC mRNA, with the greatest decrease occurring in the late afternoon just prior to the preovulatory LH surge. By contrast, treatment of ovariectomized female rats with progesterone initially decreased and then stimulated arcuate levels of POMC mRNA (171). Taken together, these results suggest that the occurrence of a preovulatory LH surge in spontaneous ovulators may result from an estrogen- and progesterone-induced removal of the inhibitory influence of β-endorphin. The progesterone-induced increase in β-endorphinergic activity may be a mechanism for terminating the LH surge. By contrast, there is no clear evidence that opioids play a role in the mating-induced preovulatory LH surge. Treatment with naloxone stimulated LH release only in the absence of circulating estradiol. Obviously, considerable estrogenic stimulation is normally present during periods of estrus in induced ovulators.

Other Neurotransmitters and Neuropeptides

The possible contributions of several other neurotransmitters to the regulation of GnRH and LH secretion have been examined in rabbits. Intravenous administration of atropine, which selectively blocks cholinergic muscarinic receptors, blocked ovulation in female rabbits, provided the drug was administered immediately after coitus (196). This finding raises the possibility that the neural circuit controlling mating-induced release of GnRH includes a cholinergic component that impinges on an adrenergic component.

The role of substance P in GnRH secretion has been studied in the rabbit (214). In the rat (215) substance-P-containing perikarya located in the arcuate nucleus project to the internal and external layers of the median eminence. In addition substance-P-containing neurons have been found to synapse with

GnRH-IR soma and dendrites in the rostral POA. Despite these anatomical links (215), the effects of substance P on LH secretion have been equivocal in rats (reviewed in 108). In rabbits (214), injection of substance P into the third ventricle augmented LH secretion in both intact and ovariectomized females. However, administration of substance P was effective only when applied during the rising, but not declining, phase of a LH peak, suggesting only a modulatory role of substance P in GnRH release. Thus, whether hypothalamic substance P participates in the postcoital preovulatory discharge of GnRH from the MBH remains unknown.

ROLE OF PITUITARY GnRH RECEPTORS IN THE PREOVULATORY LH SURGE

The amount of LH secreted in response to the MBH release of GnRH depends on the number of GnRH receptors (R) present on the plasma membrane of the pituitary gonadotropes (239). In spontaneous ovulators, including rats (3), sheep (50), and humans (221), a single intravenous injection of GnRH increases pituitary sensitivity to subsequent pulses of GnRH, presumably by increasing GnRH-R numbers (e.g., 216). As a result, consecutive short pulses of GnRH will ultimately lead to a much larger release of LH from the pituitary. By contrast, continuous infusion of GnRH leads to a decrease in GnRH-R numbers, thereby desensitizing the pituitary to the LH-releasing capacity of GnRH (51, 162). Thus, GnRH is a key regulator of pituitary GnRH-R levels and thereby the magnitude of any LH surge in spontaneous ovulators. To date, attempts (61, 151) using induced ovulators to demonstrate GnRH-induced changes in pituitary sensitivity to subsequent GnRH exposure have been unsuccessful. Donovan and ter Haar (61) found that three consecutive intravenous injections of GnRH given at hourly intervals induced repetitive LH surges in female ferrets. However, there was no sign of any modulating effect of the second or third GnRH injection on the magnitude of the resultant LH surges. Likewise, two injections of GnRH (separated by 20 or 60 min) did not increase pituitary sensitivity in the vole (*M. agrestis;* 151). However, a self-priming effect of GnRH on later pituitary sensitivity to additional GnRH stimulation may occur in the cat. As already explained, several studies suggest that repeated mating triggers episodes of GnRH release, thereby producing cumulative increments in LH secretion needed to stimulate ovulation in this species (47, 107, 238). Thus far, priming effects of GnRH on the pituitary have been studied only in the male cat. Goodrowe *et al.* (81) showed that a second injection of GnRH produced a second LH surge. However, this second response was smaller and shorter in duration than the first one, suggesting a desensitization of the pituitary by GnRH. Thus, in contrast to spontaneous ovulators in which preovulatory LH surges may partly depend upon the self-priming effect of GnRH in gonadotropes (e.g., 223), there is very little evidence for any self-priming effect of GnRH on LH secretion in induced ovulating species.

CONCLUDING REMARKS

The role of GnRH in stimulating pituitary LH secretion is well established for both spontaneously and induced ovulating species. By contrast, our understanding of the neuroendocrine mechanisms controlling GnRH synthesis and release in either spontaneous or induced ovulators is still very limited. It is not clear how sensory stimuli associated with mating reach and activate forebrain GnRH neurons in induced ovulators. In addition, how estrogen exerts its inhibitory and/or stimulatory actions on GnRH neurons has been difficult to establish in both spontaneous and induced ovulators. Finally, how GnRH neurons distributed diffusely throughout the medial basal forebrain coordinate the release of discrete pulses of GnRH into the median eminence remains largely unknown. It is possible that GnRH neurons actually form an interconnected network. Synapses have been shown between GnRH neurons (40, 41, 128, 146, 213, 240). In addition, *in vitro* studies (119) of GT1-7 cells, the immortalized mouse GnRH neuronal cell line, showed that these cells expressed the message and the protein for the GnRH-receptor. Another possibility is that GnRH release from nerve terminals is coordinated at the median eminence by other neurotransmitter or peptidergic systems such as NE and NPY (activational) or by opioids (inhibitory). Because GnRH plays such a pivotal role in reproduction, it is likely that multiple neuroendocrine mechanisms have evolved to control its release. Thus, the interruption of one particular pathway such as the one that controls estradiol-induced GnRH output, does not necessarily eliminate the possibility of GnRH release leading to a preovulatory LH surge, as demonstrated by the occurrence of mating-induced ovulation in the persistently estrous rat. The fact that the neuroendocrine mechanisms controlling both induced and spontaneous ovulation are intertwined in many mammalian species warrants further study. To what extent do the neural inputs to forebrain GnRH neurons differ between induced and spontaneous ovulators? There is strong evidence in both spontaneous and induced ovulators pointing to an activation of brainstem and midbrain noradrenergic neurons prior to the preovulatory release of GnRH (reviewed in 208). By contrast, there is no clear evidence that endogenous opioid peptides are part of the neural circuit conveying genital–somatosensory inputs to forebrain GnRH neurons in induced ovulators, probably because the neural signal for inducing a preovulatory LH surge is primarily excitatory in these species. Future studies using both induced and spontaneously ovulating species will help elucidate the neural circuitry controlling MBH GnRH release.

ACKNOWLEDGMENTS

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